

Effect of Traditional Saline Solution Concentration and Fermentation Duration on The Quality of Tilapia Sauce

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ABSTRACT

Keywords:

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The fermentation process has been known for a long time, this process basically occurs in a food. At the beginning of its appearance, the fermentation process is used by many western countries to create a food that has many flavors. In tilapia, there are carbohydrates, protein, fat, as well as a number of vitamins B3, B12, potassium, phosphorus, and selenium. For many people, besides being high in nutrition, and having a savory and sweet taste, fish sauce is also very easy to make and the cost to make it is quite cheap and doesn't take a long time. The aim of this study was to determine the effect of traditional salt concentration and the effect of fermentation time on the quality of fish sauce. The research method used was an experiment with Completely Randomized Design (CRD) as the experimental design. The treatments used were the addition of saline solution concentration and fermentation duration, each treatment was repeated 3 times. The independent variables in this study were the concentration of traditional salt used and the time of fermentation. The controlled variables in this study were *Streptococcus lactis* bacteria, pineapple fruit, temperature, pH and tilapia. The dependent variable measured was the quality of tilapia fish sauce using proximate analysis and NaCl test in percent (%). In this investigation, the optimal treatment was a 7-day fermentation with a 5% saline solution concentration (W3K1), with a protein content of 17.38%, water content of 68.66%, ash content of 8.12%, fat content of 10.85%, and 0% carbohydrate content

INTRODUCTION

Since ancient times, people have been using the fermentation process to turn many kinds of substances into processed foods. Many western nations initially employed the fermenting technique to produce food with a variety of flavors. Later advancements saw the emergence of similar technique across Asia, particularly in Indonesia. Soy sauce has a blackish color and is often used to add flavor to many foods such as soup, grilled fish, and satay (Kurniawan, 2018).

Regarding the fermentation process, one of the ingredients that is often used is fish, in this case using tilapia. Tilapia is often used because it has sufficient protein content. In one tilapia, there is a lot of protein and folic acid which is good for the body. This tilapia also contains a lot of vitamins B12, phosphorus, selenium, and potassium. In 100 grams of tilapia, there are about 128 calories, 0 grams of carbohydrates, 26 grams of protein, 3 grams of fat, and a number of vitamins B3, B12, potassium, phosphorus, and selenium.

Given these circumstances, it is not surprising that fish sauce is popular despite being high in nutrients, salty, and sweet flavors. The process of making fish sauce is likewise relatively simple, inexpensive, and quick, taking anywhere from 6 to 12 months. In simple terms, the process of making fish sauce by fermentation can be done by adding salt to be used as a microbial control compound. In the manufacture, at least a salt content of 20%-30% is required. Salt can be an agent to inhibit microbial growth, so fish sauce will last longer (has a long shelf life).

LITERATURE REVIEW

Tilapia is a freshwater fish, and is widely favored by people in Indonesia. Tilapia fish or known in Latin as *Oreochromis niloticus* is a fish that belongs to the *Tilapia nilotica* species. Basically, this tilapia fish incubates egg larvae in its mouth. At the beginning of its discovery in the mid-80s, this fish was also often referred to by the Latin name *Oreochromis niloticus* (Amri and Khairuman, 2008). In addition, tilapia is also one of three types of fish genus that are often cultivated.

The fermentation process has the main principle of activating certain microbes in order to produce the expected ingredients. When doing fermentation, at least some important factors that can affect it need to be considered. These factors are: a. microorganisms (microbial agents) b. substrate c. temperature d. oxygen e. water activity (Afrianti, 2023).

The chemical reaction that occurs in the fermentation process of making tilapia soy sauce is $C_6H_{12}O_6 \rightarrow 2 C_2H_5OOCOOH + 2 ATP$ where the substrate used in the fermentation process is glucose which will undergo a glycolysis process which produces 2 molecules of pyruvic acid. 2 molecules of lactic acid will undergo a lactic acid dehydrogenase process by enzymes so that 2 molecules of lactic acid are produced. In the glycolysis process, two ATP molecules and 2 NADH molecules are produced where 2 NADH molecules will be used in the lactic acid dehydrogenation process.

Streptococcus lactis Is an acid-producing bacterium that converts lactose into lactic acid, round shape with positive chain, no spore, can grow anaerobically. This bacterium is homofermentative, optimum temperature 65°C, minimum pH 4.3-4.8 (Supardi, et al, 2015).

This analysis aims to determine the chemical composition of a food ingredient. Proximate analysis is one of the analyses that does not require many

technologically advanced instruments. One of the main results of this analysis is the TDN (total digestible nutrients) value for food evaluation. However, this analysis has 12 major drawbacks, such as the lack of an explanation of the digestibility and texture of food ingredients (Suparjo, 2010).

Hedonic test is this test is an organoleptic sensory analysis. In essence, this test is useful to see the difference between the quality of a product based on its nature. In addition, this test is also useful for reviewing the level of allure of a product with the criteria of dislike, dislike, like, like very much (Tarwendah, 2017). Another implication that will be obtained from this test is being able to control the quality of a product (Permadi, 2018).

METHOD

Equipment used in this study are areporcelain cup, cruss pliers, triangular wire, electric scales, oven, exicator, Bunsen, electric furnace, 100 cc Kjedral flask, Kjedral flask heater, measuring cup, 250 cc measuring flask, 100 cc and 1000 cc erlenmeyer, marcam steel tool, flask filter, Soxhlet flask, reflux cooler, Erlenmeyer suction, Buchner funnel and spatula. The material used in this study was test fish, tilapia (*Oreochromis niloticus*). Kjeldahl tablets (Kjeldahl tablets contain chemicals like sodium hydroxide, selenium, and sodium sulfate that help break down the sample during the digestion process. The digestion turns the nitrogen into ammonia, which can then be measured); concentrated H_2SO_4 ; NaOH is 40%; boric acid; methyl peacock and cresol green brim; H_2SO_4 measuring 0.01 and 0.3; NaOH size 1.5; HCl 0.3; Acetone; H_2O ; Aquades; Streptococcus lactis bacteria; and 200 g of pineapple.

The research method used is the experimental method. Experimental research is a research method carried out by testing the influence of one or more variables with other variables (Untari, 2018). The study was conducted with the aim of determine the causal relationship of one or all of the treatments given and compared with controls The design used in this study used a completely randomized design (CRD). This study used three treatments of salt content and four treatments of fermentation duration with three replications. Analysis of variance (ANOVA) was then used to look at the relationship between each variable to see whether or not there were significant results in each treatment for each variable.

There are three main stages to the research process. The first is the preparation of materials and tools. This step begins with the preparation of all the tools and materials that were previously determined. The fish is then utilized entirely, save for the bones, but is destroyed prior to moving on to the following stage. As a result, the next step is simpler to accomplish, and as the fish has already been ruined, the fermentation process goes more quickly. Stages of Hydrolysis and Fermentation is the second. The enzyme bromelain, which comes from pineapple fruit, is added up to 50 grams at this stage. The pineapple fruit that has previously

been combined with tilapia is where the enzyme is extracted. When they were done, the researchers added lauric salt to it. The combined salts had concentrations of 5 percent, 10 percent, and 15 percent. In addition, 40 ml of 5.8×10^8 *Streptococcus lactis* bacteria were introduced. The fermentation process took two, five, seven, and 10 days at a temperature of 37°C.

The next stage is Proximate Testing. The first stage is the water content test (SNI-01-23354.2-2015), the second stage is the ash content test (SNI 2354.1:2010), the third stage is the protein content test (SNI-01-2354.4-2006), the fourth stage is the fat content test (SNI-01-2354.3-2017), and the last stage is the carbohydrate content using the by-difference method. The next stage is the NaCl test. This test is carried out by spectrophotometry. A useful way to see the presence of an atom or another element. To find out this, a test was carried out with the main step being to compare the amount of energy emitted from the radiation source⁴. In this regard, in this test an analysis of bentonite using AAS (Atomic Absorption Spectrophotometry) was also carried out to determine the content of Na, Ca, Mg, and Fe. The last step was hedonic testing on 25 unskilled persons. Then the panelists were asked to rate the content of preference on 3 criteria, namely aroma, taste, and color.

RESULT AND DISCUSSION

The results of the analysis of variance (ANOVA) based on the smallest real object test (BNT) showed a significant difference ($P < 0.05$) in each treatment. The results of the calculation of the proximate analysis and NaCl contents can be seen in table 1

Table 1. Results of the proximate analysis test and the NaCl level test

Sample	Protein Level (%±SD)	Fat Level (%±SD)	Carbohydrate Level (%±SD)	Water Level (%±SD)	Ash Level (%±SD)	NaCl Level (%±SD)
W1K1	12.87 ± 1.00	0.4 ± 0.08	4.35 ± 0.41	76.45 ± 0.78	5.93 ± 0.39	6.57 ± 0.43
W1K2	2.74 ± 0.53	9.6 ± 0.46	6.87 ± 0.43	73.41 ± 0.52	7.38 ± 0.52	10.11 ± 0.52
W1K3	7.18 ± 0.14	4.3 ± 0.32	0.17 ± 0.06	76.41 ± 0.47	11.94 ± 0.56	11.72 ± 0.56
W2K1	9.28 ± 0.38	2.84 ± 0.23	3.52 ± 0.18	78.12 ± 0.29	6.24 ± 0.69	5.86 ± 0.11
W2K2	4.04 ± 0.41	5.32 ± 0.45	7.73 ± 0.63	73.05 ± 0.43	9.86 ± 0.34	10.14 ± 0.62
W2K3	6.63 ± 0.53	2.87 ± 0.05	4.85 ± 0.19	69.67 ± 0.65	15.98 ± 0.19	14.12 ± 0.63
W3K1	17.38 ± 0.21	10.85 ± 0.86	0 ± 0	68.66 ± 1.23	8.12 ± 0.02	7.40 ± 0.55
W3K2	7.1 ± 0.44	8.52 ± 0.58	1.15 ± 0.19	73.45 ± 0.19	9.78 ± 0.19	9.68 ± 0.07
W3K3	11.25 ± 0.53	0.74 ± 0.11	2.36 ± 0.27	70.22 ± 0.43	15.42 ± 0.10	14.06 ± 0.39
W4K1	17.16 ± 0.67	1.34 ± 0.30	1.38 ± 0.23	73.30 ± 0.92	6.82 ± 0.20	7.10 ± 0.51
W4K2	6.01 ± 0.70	1.19 ± 0.10	6.23 ± 0.65	77.13 ± 1.11	9.44 ± 0.18	9.47 ± 0.67
W4K3	9.55 ± 0.69	0.61 ± 0.05	1.02 ± 0.12	76.13 ± 0.52	12.69 ± 0.70	11.76 ± 0.48

Fermentation of tilapia soy sauce in this study was carried out by adding pineapple fruit as a source of bromelain enzyme which is useful to help the hydrolysis process. Bromelain enzyme is a proteolytic or protease type enzyme that

catalyzes the breaking of peptide bonds in proteins resulting in an increase in protein content (Nur et al., 2017). The results of research by Prasetyo et al. (2022) showed that the greater the concentration of bromelain enzyme used, the higher the protein content. While the results of research by Iskandar & Widyasrini (2019) showed that the more the addition of pineapple fruit extract containing bromelain enzyme, the greater the nitrogen content in lemuru fish sauce will be obtained until a certain time limit, and this indicates the occurrence of hydrolysis which is even greater.

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The results of the proximate test showed that the highest protein content was obtained in the sample with 5% salt content and fermentation time for 7 days, which amounted to 17.38%. This happened because the bromelain enzyme at the beginning of fermentation was still in the process of hydrolyzing the peptide bonds in tilapia so that not much had been solved into amino acids. The process continues until the highest amino acid levels are obtained on day 7 of fermentation. These results are in line with the results of research by Khotimah & Soetikno (2016) and Kurniawan (2018) who found that in the fermentation of soy sauce, each made from sawah snails and catfish; by adding bromelain enzyme, the highest protein content was obtained on day 7 of fermentation for all samples. The highest protein content of 17.38, in this study is not much different from the results of research by Astuti, et al. (2012) who obtained the highest protein content of 17.57% in tuna soy sauce.

When viewed from the data trend, with the same salt on the 2nd day of fermentation, the protein content was 12.87%, then dropped to 9.28% on the 5th day of fermentation, then rose dramatically to 17.38% on the 7th day, and slightly dropped on the 10th day of fermentation to 17.16%. Meanwhile, when compared to other samples using 10% and 15% salt, all samples with 5% salt had higher protein levels at all fermentation times. This happens because of the higher salt concentration so that the amount of protein that is solved into amino acids decreases. These results are in line with the results of research by Kurniawan (2018) who found that the higher the salt, the lower the protein content in catfish soy sauce, both for fermentation times of 1, 3, and 7 days.

The decrease in protein content from day 2 to day 5 in the treatment of 5% and 15% salt addition may be caused by a decrease in bacterial activity due to the reduced amount. Hutkins (2019) stated that proteolysis (protein breakdown into polypeptides or amino acids) of fish protein in the fermentation process is caused by proteolytic activity carried out by microbes (bacteria). Bacteria develop in 4 (four) phases, namely lag, log, stationary, and death phases (Behera et al., 2019). Days 2 and 5 in this study are likely for bacteria that are still in the lag phase, which is when they are still adapting to their environment. In this phase, the bacteria did not experience growth, and the number might even decrease when some of them died because they were unable to adapt (Behera et al., 2019), so that the protein levels dropped.

The proximate test results showed that the highest fat content was obtained by the sample with 5% salt concentration and 7 days fermentation time, which amounted to 10.85%. The data trend showed an increase from day 2 to day 7, then decreased on day 10. This may be due to the fact that the fat content increased along with the increasing content of lipoproteins (fat-bound protein molecules) that dissolve and are deposited between collagen-collagen. These results are in line with the results of research by Khotimah & Soetikno (2016) who found that the fat content in fermented soy sauce made from rice paddy snails showed an upward trend from day 1 to day 7, and then decreased on day 10.

The proximate test results showed that the highest carbohydrate content was obtained in the sample with 10% salt concentration and 5 days fermentation time, which amounted to 7.73%. The data trend showed an increase on day 5, then decreased on day 7, and increased again on day 10. When viewed on the graph in Figure 8, there are extreme values and significant differences with other values. This is because in this study the measurement of carbohydrate content was carried out using the by difference method, which involves the calculation of water content, ash content, protein content and fat content.

The proximate test results also showed that the highest ash content was obtained in the sample with 15% salt concentration and 5 days fermentation time, which amounted to 15.98%. The data trend was from day 2, increased on day 5, then decreased on day 7, and decreased again on day 10. When compared between salt concentrations, the lowest ash content was found in the sample with 5% salt, and the highest in the sample with 15% salt. This is probably because the higher the salt, the higher the mineral content so that the ash content is also higher. These results are in line with research by Sari et al. (2018) which stated that ash content increases as salt content increases because salt causes mineral content in fermented soy sauce products to increase, causing ash content to also increase.

The proximate test results in this study showed that the highest water content was found in the sample with 5% salt concentration and 5 days fermentation time, which amounted to 78.12%. This value is higher than the results

of research by Siahaan, et al. (2017) which found that the water content in the fermentation of tuna soy sauce with the addition of pineapple juice ranged from 69.14-71.71%. In the sample with 5% salt concentration, the data trend shows, starting from day 2, the data rises on day 5, then drops on day 7, and rises again on day 10. In general, the pattern is decreasing. This pattern is in line with the results of research by Khotimah & Soetikno (2016) which showed the same trend in the results of the proximate test of water content in rice snail soy sauce. This is because water is absorbed by salt, so the greater the salt concentration, the less water content. In addition, according to Tungkawachara et al. (2003) in making fish sauce, the longer the fermentation time, the water content will decrease.

CONCLUSION

The addition of conventional saline solution concentration and fermentation duration had an effect on the quality of tilapia fish sauce, according to the findings. In this investigation, the optimal treatment was a 7-day fermentation with a 5-percent saline solution concentration (W3K1), with a protein content of 17.3. As a result of the research results, further research is needed in terms of the chemical physics of the fish sauce products produced so that the results obtained can be more specific because from this study only the general chemical content is obtained, not specific 8%, water content of 68.66%, ash content of 8.12%, fat content of 10.85%, and 0% carbohydrate content.

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